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A copy of the following refrence(s) is requested :

1. ANSWER 6 OF 7 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1982:300038 BIOSIS
DOCUMENT NUMBER: BA74:72518
TITLE: OZONE INDUCED FORMATION OF O O' **DI TYROSINE** CROSS LINKS IN PROTEINS.
AUTHOR(S): VERWEIJ H; CHRISTIANSE K; VAN STEVENINCK J
CORPORATE SOURCE: SYLVIUS LAB., DEP. MED. BIOCHEMISTRY, 'WASSENAARSEWEG 72, 2333 AL LEIDEN.
SOURCE: **BIOCHIM BIOPHYS ACTA, (1982) 701 (2), 180-184.**

2. TITLE: CHEMICAL NATURE OF MONOGENEAN SCLERITES PART 1 STABILIZATION OF CLAMP **PROTEIN** BY FORMATION OF **DI TYROSINE.**
AUTHOR(S): RAMALINGAM K
SOURCE: PARASITOLOGY, (1973) 66 (1), 1-7.
CODEN: PARAAE. ISSN: 0031-1820.

TITLE: CD and proton NMR studies on the side-chain conformation of tyrosine derivatives and tyrosine residues in di- and tripeptides
AUTHOR(S): Juy, Michel; Lam Thanh Hung; Fermandjian, Serge
CORPORATE SOURCE: Dep. Biol., Cent. Nucl. Stud., Gif-sur-Yvette, 91191, Fr.
SOURCE: **International Journal of Peptide & Protein Research (1982), 20(4), 298-307**

3. Journal of the American Chemical Society (1985), 107(3), 659-66

4. **BIOCHEMICAL JOURNAL,** (2003 Mar 1) 370 (Pt 2) 729-35.

5. Salt-**stabilized protein** formulation

SOURCE: **Research Disclosure (1995), 370, 56-7**

Thank you !

Tekchand Saidha
Primary Examiner
Art Unit 1652, CM1, Room No. 10D05
Mail Box 10D01
(703) 305-6595

Conclusions

The present ^{13}C CPMAS investigation provides an important complement t the surface chemical results[1] in delineating adsorbate–alumina interactions for a highly active heterogeneous rganoactinide catalyst. While the chemical results provide evidence for structures such as E–H, the surface NMR studies strengthen this formulati n and, moreover, indicate that transfer of methyl groups from the actinide to surface aluminum sites has also occurred. To our knowledge, the latter result represents the first direct observation of this type of process for a supported catalyst. It is likely that such chemistry is not restricted to organoactinides and that similar processes not only occur but are important in numerous other catalytic systems comprised of relatively polar metal alkyls and high surface area metal oxides.[43,44]

Acknowledgment. We are grateful to the Department of Energy for support of this research under Contract DEAC 02-81ER10980. High-field solid-state ^{13}C NMR spectra were perf rmed by the Colorado State University Regional NMR Center, funded by National Science Foundation Grant CHE-8208821. We thank H. A. Stecher f r several preliminary NMR spectra, C. M. Fendrick for a sample of $Cp'_2Th(CH_3)(CH_2CMe_3)$, and K. G. Moloy for a sample of $Cp'_2Th(CH_3)[OCH(t\text{-}Bu)_2]$. We thank Dr. Mark Delaney of Dow Chemical Co. for poly(methylaluminoxane) samples and Professor R. L. Burwell, Jr., for stimulating discussions.

Registry No. $Cp'_2Th(CH_3)_2$, 67506-90-5; $Cp'_2Th(^{13}CH_3)_2$, 94138-24-6; $[Cp'_2Th(\mu\text{-}H)H]_2$, 67506-92-7; $Cp'_2Th(CH_3)(CH_2CMe_3)$, 94138-25-7; $Cp'_2Th(CH_3)Cl$, 79301-20-5; $Cp'_2Th(CH_3)[OCH(t\text{-}Bu)_2]$, 94138-26-8; $Cp'_2Th(CH_3)(OCMe_3)$, 79301-34-1; Al_2O_3, 1344-28-1; $[(CH_3)AlCl_2]_2$, 15171-31-0.

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Copper(II) Complexes of Tyrosine-Containing Dipeptides. Effects of Side-Chain Groups on Spectral and Solution Chemical Properties and Their Structural Implication

Osamu Yamauchi,* Kiyokazu Tsujide, and Akira Odani

Contribution from the Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920, Japan. *Received July 23, 1984*

Abstract: With a view to obtaining information of the structures and stabilities of the Cu(II) complexes of tyrosine (Tyr)-containing dipeptides, spectroscopic and potentiometric studies have been carried out with the peptides to L-Tyr-X, where X refers to L-/D-alanine, -arginine, -Tyr, -tryptophan (Trp), -histidine (His), L-phenylalanine (Phe), L-/D-glutamic acid. The complex species and their stability constants have been determined by potentiometric titrations at 25 °C and $I = 0.1$ (KNO_3). All the peptides react with Cu(II) in the manner analogous to L-tyrosylglycine (L-Tyr-Gly), but the deprotonation of the peptide NH group is affected by the C-terminal side-chain groups. The dipeptides except L-Tyr-L-/D-His form at pH 8–11 a dimeric species, the maximum distribution of which occurs at pH ~9.5 in the 1:1 Cu(II)–peptide systems with the dimer accounting for as much as 80% of the total Cu(II) in 5 mM Cu(II)-L-Tyr-L-Trp. The constants for 2(monomer) = dimer equilibria are in the range 2.04–3.70 log units. The absorption spectra of the 1:1 systems (~2 mM) exhibit a d–d peak at 610–630 nm (ϵ 90–150) at pH >6 and in the presence of the dimeric complex an additional peak at ~380 nm (ϵ 260–720), whose assignment to the charge transfer between Cu(II) and the phenolate group has been confirmed by the resonance Raman spectra of the isolated complexes, $[Cu(L\text{-}Tyr\text{-}Gly)]\cdot 0.5H_2O$ and $Na_2[Cu_2(L\text{-}Tyr\text{-}Gly)_2]\cdot 7.5H_2O$. While the circular dichroism (CD) spectral magnitudes in the d–d region for the Cu(II)–dipeptide complexes with a C-terminal aliphatic amino acid are an additive function of those exhibited by the component amino acid complexes irrespective of the diastereoisomerism of the peptides, remarkable CD magnitude anomaly was observed for the active (L–L) peptides with a C-terminal aromatic amino acid, L-Tyr-L-X (X = Tyr, Trp, and Phe). The anomaly is diastereospecific and strictly coincident with the dimer formation, which is taken to imply distortion by the dimeric structure of the C-terminal side-chain orientation favoring the Cu(II)–aromatic ring interaction.

Tyrosine constitutes the N-terminus of endogenous analgesic peptides such as enkephalin[1] and endorphin[2] isolated from brain and is considered to be essential for their activity.[3] An analgesic dipeptide, L-tyrosyl-L-arginine (kyotorphin), was later isolated from bovine brain by Takagi et al.,[4] which again indicates the importance of the N-terminal tyrosyl residue. Of the main biological transition metals, copper(II) is probably the most effective in binding small peptides at physiological pH,[5] and since the copper content is high especially in the synaptosomal fraction of brain which abounds in enkephalin and kyotorphin,[6] interactions of copper(II) with these opioid peptides may have physiological

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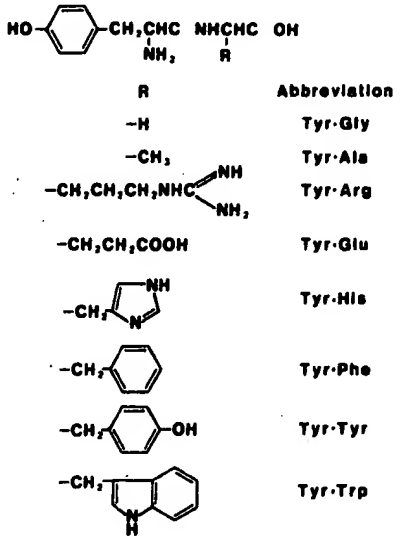
Chart I



signficance in the analgesic activity.

Gairin et al. found by spectroscopic methods formation of stable Cu(II)–enkephalin complexes and proposed the structures in solution, where the phenolate oxygen is not involved in coordination.[7] On the other hand, Hefford and Pettit disclosed that L-tyrosylglycine and L-tyrosyl-L-/D-leucine in the alkaline region form a dimeric copper(II) complex exhibiting an absorption band centered at 375 nm ascribable to the phenolate–Cu(II) bonding.[8] Since the N-terminal tyrosine phenol moiety and the neighboring side-chain groups of opioid peptides and possibly their copper(II) complexes appear to have a crucial effect on the physiological activity, we have been interested in the chemical properties of the copper(II) complexes of simple tyrosine peptides in solution and their possible relevance to the physiological activity of the opioid peptides. In this connection, the steric effects of the dimer formation on the side-chain conformations are particularly intriguing, since they may afford information of the conformations in monomeric copper(II) complexes of tyrosine peptides in solution. We here report the structures, solution equilibria, and optical properties of the copper(II) complexes of tyrosine dipeptides containing charged aliphatic or uncharged aromatic side chains (Chart I) with emphasis on the phenolate–copper(II) interactions, remarkable circular dichroism (CD) spectral changes associated with formation of the dimers, and their structural implication.

Experimental Section

Materials.[9] L-Tyr-Gly, L-Tyr-L-Ala, L-Tyr-L-Glu, Gly-L-Tyr, Gly-L-Trp, L-Tyr-L-Phe, L-Phe-Gly, L-Phe-L-Tyr, and Gly-L-Ala were purchased from Sigma and Gly-L-Phe from the Protein Research Foundation. L-Tyr-Gly and Gly-L-Tyr were purified before use by DEAE cellulose column chromatography and recrystallization, respectively. L-Tyr-L-Arg, L-Tyr-D-Arg, and Gly-L-Arg were generous gifts from Daiichi Pharmaceutical Co. The other dipeptides were prepared in our laboratory and checked by elemental analysis and thin-layer chromatography (the melting point (°C, uncorrected) and specific rotation $[\alpha]_D$ (concn) in water at 19.3 °C are given in this order): L-Tyr-D-Ala, 200, +119.1° (*c* 0.5); L-Tyr-D-Glu·2H$_2$O, 151–154, +90.8° (*c* 0.5); L-Tyr-L-His·HCl, 210, +34.0° (*c* 0.4); L-Tyr-D-His·H$_2$O, 186–187, +24.7° (*c* 0.2); L-Tyr-L-Tyr·2H$_2$O, 161–163, +28.7° (*c* 0.3); L-Tyr-D-Tyr·H$_2$O, 171–172, −36.3° (*c* 0.3); L-Tyr-L-Trp·H$_2$O, 175–176, +16.0° (*c* 0.4 in 1 M HCl); L-Tyr-D-Trp·4.5H$_2$O, 160–162, +23.8° (*c* 0.4 in 1 M HCl); L-Ala-L-Arg·HCl, 239–240, +8.4° (*c* 0.5); Gly-L-His·HCl, 187–188, +28.0° (*c* 0.3).

All chemicals used were of reagent grade or highest grade available, and distilled and deionized water was used throughout.

Syntheses of Copper(II) Complexes. [Cu(L-Tyr-Gly)]·0.5H$_2$O. To a solution containing L-Tyr-Gly (0.24 g, 1.0 mmol) and NaOH (0.08 g, 2 mmol) was added Cu(NO$_3$)$_2$·3H$_2$O (0.24 g, 1.0 mmol) with stirring at room temperature. The blue crystals which separated from the solution were collected, washed with a small amount of ethanol, and dried in the open air. Anal. Calcd for $C_{11}H_{12}N_2O_4Cu\cdot0.5H_2O$: C, 42.82; H, 4.24; N, 9.08. Found: C, 42.83; H, 4.18; N, 9.01.

Na$_2$[Cu$_2$(L-Tyr-Gly)$_2$]·*n*H$_2$O (*n* = 7.5, 10.5). An aqueous solution containing L-Tyr-Gly (0.24 g, 1.0 mmol), Cu(NO$_3$)$_2$·3H$_2$O (0.24 g, 1.0 mmol), and NaOH (0.12 g, 3 mmol) was stirred for 1 h at room temperature and concentrated in vacuo. The green crystals which separated from the solution were collected, washed with ethanol, and recrystallized from water containing a small amount of NaOH. Anal. Calcd for $C_{22}H_{22}N_4O_8Cu_2Na_2\cdot10.5H_2O$: C, 31.73; H, 5.21; N, 6.73. Found: C, 31.80; H, 5.08; N, 6.80. When dried in vacuo, the crystals lost water molecules to give Na$_2$[Cu$_2$(L-Tyr-Gly)$_2$]·7.5H$_2$O. Anal. Calcd for $C_{22}H_{22}N_4O_8Cu_2Na_2\cdot7.5H_2O$: C, 33.94; H, 4.79; N, 7.20. Found: C, 33.93; H, 4.54; N, 7.18.

[Cu(L-Tyr-L-His)]·0.5H$_2$O. Aqueous solutions of L-Tyr-L-His·HCl·0.5H$_2$O (0.20 g, 0.55 mmol) and Cu(ClO$_4$)$_2$·6H$_2$O (0.20 g, 0.55 mmol) were mixed to make the final volume 150 mL. The solution was neutralized (pH ~7) and kept at room temperature, when violet crystals separated. Anal. Calcd for $C_{15}H_{16}N_4O_4Cu\cdot0.5H_2O$: C, 46.33; H, 4.41; N, 14.41. Found: C, 46.88; H, 4.24; N, 14.78.

pH Titrations. Carbonate-free 0.1 M KOH was prepared under N$_2$ and standardized against standard potassium hydrogen phthalate (Merck DIN 19226). Copper(II) nitrate (0.01 M) was standardized by chelatometry with metallic zinc (JIS primary standard) as standard. pH values were measured with an Orion Research 901 ion meter equipped with an Orion 91-01-00 glass electrode and a 91-02-00 double junction reference electrode. Calibration of the meter was made with NBS standard buffer solutions (4.008, 7.413, and 9.180 at 25 °C). The difference between the pH meter reading pH$_M$ and −log [H], where [H] denotes the hydrogen ion concentration, was determined as reported previously to be 0.067 at I = 0.1 (KNO$_3$).[10] The apparent ion product of water $pK_w' = pH_M - \log [OH]$, where [OH] is the hydroxide ion concentration, was 13.96 at I = 0.1. Solutions of ligands only and of Cu(II) and a ligand with the molar ratio of 1:1 or 1:2 were titrated at 25 ± 0.05 °C under N$_2$. Reproducibility of the results was checked by repeated titrations.

Calculation of Equilibrium Constants. The stability constants are expressed as overall constants, β_{pqr}, for species containing Cu(II), ligand L, and proton H in the molar ratio of p, q, and r, respectively (charges are omitted for simplicity)

$$pCu + qL + rH \overset{\beta_{pqr}}{\rightleftharpoons} Cu_pL_qH_r \quad (1)$$

$$\beta_{pqr} = \frac{[Cu_pL_qH_r]}{[Cu]^p[L]^q[H]^r} \quad (2)$$

where a negative value of r refers to deprotonation from the complex. The log β_{pqr} values were calculated by the method of nonlinear least squares with the computer program MINIQUAD[11] by a FACOM M-170F computer at the Kanazawa University Computation Center. The R factors were in the range 0.04–0.35%.

Spectroscopic Measurements. Absorption spectra were measured for Cu(II)–ligand systems with the total Cu(II) concentrations of 0.1–5 mM at various pH values in the range 250–800 nm with a Union Giken SM-401 high-sensitivity recording spectrophotometer, and the CD spectra were measured in the range 300–800 nm with JASCO J-20 and J-500C spectropolarimeters. Infrared absorption and resonance Raman spectra were recorded with a JASCO A-202 infrared spectrophotometer and a JASCO R-800 laser Raman spectrophotometer, respectively.

Results

Solution Equilibria. Typical titration curves for the proton–ligand and Cu(II)–ligand systems are shown in Figure 1. The log β_{01r} values for the proton–ligand complexes calculated from the titration data are summarized in Table I. The reactions of Cu(II) with the tyrosine-containing dipeptides except L-Tyr-L-/

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(8) Hefford, R. J. W.; Pettit, L. D. *J. Chem. Soc., Dalton Trans.* **1981**, 1331–1335.

(9) Abbreviations of dipeptides are as follows: Tyr-Gly, tyrosylglycine; Tyr-Ala, tyrosylalanine; Tyr-Glu, tyrosylglutamic acid; Tyr-Leu, tyrosylleucine; Gly-Tyr, glycyltyrosine; Gly-Trp, glycyltryptophan; Tyr-Phe, tyrosylphenylalanine; Phe-Gly, phenylalanylglycine; Phe-Tyr, phenylalanyltyrosine; Gly-Ala, glycylalanine; Tyr-Arg, tyrosylarginine (kyotorphin); Gly-Arg, glycylarginine; Tyr-His, tyrosylhistidine; Tyr-Tyr, tyrosyltyrosine; Tyr-Trp, tyrosyltryptophan; Ala-Arg, alanylarginine; Gly-His, glycylhistidine.

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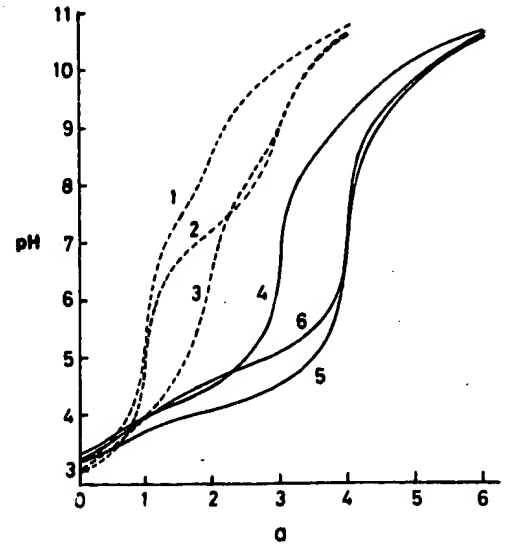


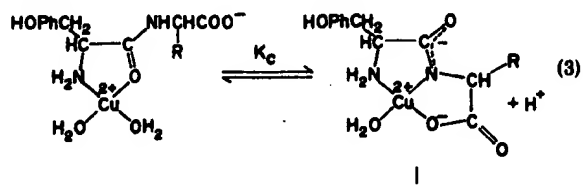
Figure 1. Typical titration curves for proton–ligand (2 mM) and 1:1 Cu(II)–ligand (1 mM) systems. Curves: 1, L-Tyr-L-Tyr; 2, L-Tyr-L-His; 3, L-Tyr-D-Glu; 4, Cu(II)–L-Tyr-L-Tyr; 5, Cu(II)–L-Tyr-L-His; 6, Cu(II)–L-Tyr-D-Glu. a = moles of KOH added per mole of ligand.

Table I. Stability Constants for Proton–Dipeptide Complexes at 25 °C and I = 0.1 (KNO_3)[a]

dipeptide	stability constants			
	log β_{011}	log β_{012}	log β_{013}	log β_{014}
L-Tyr-Gly	9.967 (2)	17.587 (3)	20.736 (5)	
L-Tyr-Gly[b]	9.926 (2)	17.613 (3)	20.767 (4)	
L-Tyr-L-Ala	9.975 (1)	17.481 (2)	20.859 (3)	
L-Tyr-D-Ala	9.790 (3)	17.447 (5)	20.501 (8)	
L-Tyr-L-Arg	9.820 (1)	17.059 (3)	20.146 (4)	
L-Tyr-D-Arg	9.818 (3)	17.442 (5)	20.256 (9)	
L-Tyr-L-Glu	10.093 (1)	17.778 (1)	22.255 (1)	25.421 (2)
L-Tyr-D-Glu	10.167 (1)	18.020 (2)	22.665 (3)	25.517 (4)
L-Tyr-L-His	10.001 (2)	17.632 (3)	24.277 (3)	26.957 (5)
L-Tyr-D-His	9.838 (1)	17.603 (5)	24.245 (6)	26.59 (1)
L-Tyr-L-Tyr	10.424 (2)	20.120 (1)	27.432 (3)	30.665 (4)
L-Tyr-D-Tyr	10.538 (2)	20.269 (1)	28.012 (3)	30.942 (4)
L-Tyr-L-Trp	9.910 (4)	17.277 (5)	20.787 (8)	
L-Tyr-D-Trp	10.046 (3)	17.960 (5)	21.237 (9)	
L-Tyr-L-Phe	10.154 (2)	17.526 (5)	20.661 (7)	
Gly-L-Tyr	10.037 (3)	18.157 (4)	21.138 (7)	
Gly-L-Tyr[b]	10.133 (3)	18.335 (3)	21.390 (4)	
L-Phe-L-Tyr	9.961 (1)	17.155 (1)	20.412 (2)	
L-Ala-L-Arg	7.946 (1)	10.939 (2)		

[a] Values in parentheses denote estimated standard deviations. [b] Taken from ref 8.

D-His proceed in the same way as with tyrosylglycine,[8] and formation of a dimeric species, which was detected in the pH range 8–10.5 and determined to be $Cu_2L_2H_{-2}$ for L-Tyr-Gly and L-Tyr-L-/D-Leu, was confirmed for various dipeptides with the N-terminal tyrosyl residue irrespective of the C-terminal side chain except L-Tyr-L-/D-His (Table II). The species distribution calculated from the stability constants as a function of pH are depicted for 5 mM solutions of the 1:1 Cu(II)–L-Tyr-L-/D-Trp systems in Figure 2, which shows that the complex with the coordination mode **1** is the predominant species at pH 5–8 and that the further deprotonated monomeric and dimeric complexes coexist in the alkaline region with the distribution maxima occurring at the same pH (~9.5). The dimer is present in a greater



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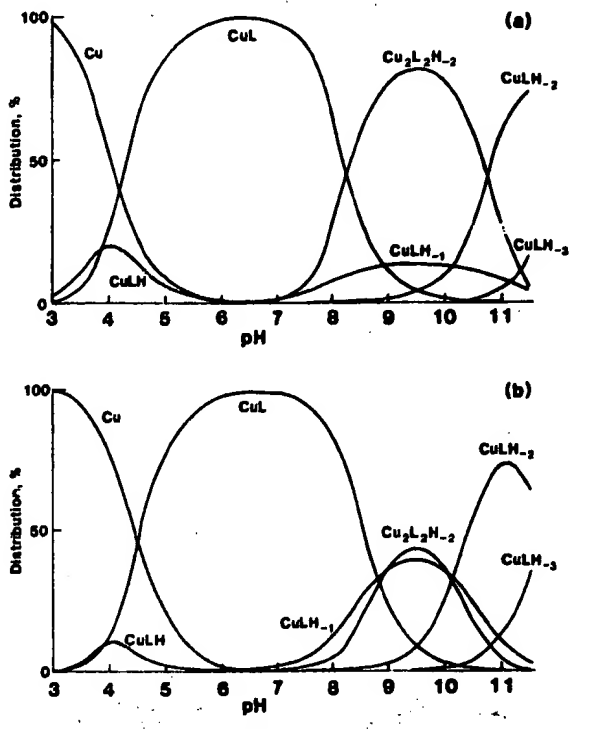

Figure 2. Species distributions in 1:1 Cu(II)–L-Tyr-L-Trp (a) and 1:1 Cu(II)–L-Tyr-D-Trp (b) as a function of pH. Calculated from the stability constants listed in Tables I and II. Concentrations: Cu(II), 5 mM; L-Tyr-L-/D-Trp, 5 mM.

amount in the systems with the active (L–L) peptides than with the meso (L–D) peptides, reflecting higher stability constants (log β_{22-2} and log β_{220}) for the active complexes (Table III). On the other hand, no dimer was detected by the equilibrium study at pH <10 in the systems with L-Tyr-L-/D-His which form complexes similar to the Cu(II)–glycylhistidinate complex.[12]

Table II reveals that there is another stereoselectivity in the Cu(II)–dipeptide complex formation; for the diastereomeric Cu(II)–dipeptide systems, those with the active peptides have greater stability constants for the complexes **1** (log β_{110} for Tyr-Ala, Tyr-Arg, Tyr-Trp, and Tyr-His and log β_{111} for Tyr-Tyr), which coincides with the observations with the other Cu(II)–dipeptide systems.[13] This is mainly due to facile deprotonation of the peptide NH group in the active peptide complexes, whose deprotonation constants pK_c (= −log K_c, eq 3), given by the differences log β_{112} − log β_{111} for Tyr-Tyr and log β_{111} − log β_{110} for the rest of the peptides, are smaller than those for the meso peptide complexes.

Absorption and CD Spectra. The 1:1 Cu(II)–dipeptide systems have the d–d band centered at ~630 nm at neutral pH where the deprotonated species **1** predominates (Table IV). The d–d peak at 586 nm exhibited by Cu(II)–L-Tyr-L-/D-His at pH 9.0 indicates additional nitrogen coordination. As has been observed for L-Tyr-Gly and L-Tyr-L-/D-Leu,[8] the systems with the N-terminal tyrosine irrespective of the C-terminal residue exhibit absorption and CD peaks at 380–400 nm at pH 8–11 ascribable to the charge transfer (CT) between Cu(II) and the phenolate group (Table V), the intensity being maximum at pH ~9.5 and coincident with the distribution of the dimeric species (Figure 2). The intensity is dependent on the concentrations of solutions, which supports that the band is due to the dimer (Figure 3).

At pH <9 the 1:1 Cu(II)–dipeptide systems have a single CD peak in the region 600–700 nm and, when dimers are formed at pH 8–11, a negative peak at 380–400 nm with additi nal peaks in the region 400–600 nm. L-Tyr-Gly and the meso peptides show

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Table II. Stability Constants for Cu(II)–Dipeptide Complexes at 25 °C and I = 0.1 (KNO_3)[a]

dipeptide	stability constants, log β_{pqr}, for pqr = 112	111	110	11-1	11-2	11-3	220	22-1	22-2	$-\log K_c$	$\log K_D$
L-Tyr-Gly		14.84 (3)	11.383 (2)	2.45 (1)	−7.799 (4)				7.34 (4)	3.46	2.44
L-Tyr-Gly[b]		15.18 (2)	11.409 (1)	2.32 (1)	−7.890 (6)				7.26 (6)	3.77	2.62
L-Tyr-L-Ala		15.04 (3)	11.623 (2)	2.67 (2)	−7.617 (5)				7.66 (6)	3.42	2.32
L-Tyr-D-Ala		14.70 (3)	11.217 (1)	2.11 (1)	−8.044 (6)				6.78 (6)	3.49	2.56
L-Tyr-L-Arg		14.38 (6)	11.335 (2)	2.58 (2)	−7.434 (6)				7.30 (9)	3.05	2.14
L-Tyr-D-Arg		14.52 (6)	10.849 (2)	1.94 (2)	−8.010 (7)				6.71 (5)	3.65	2.83
L-Tyr-L-Glu		16.615 (1)	11.824 (0)	2.255 (4)	−7.818 (1)			16.73 (1)	7.991 (5)	4.79	3.48
L-Tyr-D-Glu		16.431 (7)	11.716 (2)	2.29 (6)	−7.74 (1)			16.6 (1)	7.7 (1)	4.72	3.12
L-Tyr-L-Tyr	25.09 (3)	21.907 (1)	13.113 (8)	3.205 (5)	−7.360 (5)		28.94 (3)	19.57 (2)		3.18	2.72
L-Tyr-D-Tyr	25.37 (2)	21.715 (1)	12.898 (4)	2.914 (3)	−7.683 (2)		27.83 (5)	18.41 (2)		3.65	2.03
L-Tyr-L-Trp		15.570 (9)	11.724 (1)	2.793 (7)	−7.319 (2)	−19.44 (3)			9.277 (8)	3.85	3.70
L-Tyr-D-Trp		15.505 (9)	11.656 (0)	2.823 (5)	−7.325 (1)	−19.06 (1)			8.08 (4)	3.85	2.44
L-Tyr-L-Phe		15.18 (1)	11.984 (1)	3.118 (4)	−7.495 (3)	−19.30 (4)			8.36 (3)	3.20	2.12
L-Tyr-L-His	22.56 (4)	19.103 (7)	15.141 (4)	5.962 (5)	−4.387 (5)						
L-Tyr-D-His		18.374 (6)	13.871 (2)	4.801 (4)	−5.218 (3)						
Gly-L-Tyr		15.88 (3)	11.865 (4)	2.728 (9)	−7.64 (1)			16.91 (7)		4.02	
Gly-L-Tyr[b]		16.127 (8)	12.096 (1)	3.029 (3)	−7.70 (1)					4.03	
L-Phe-L-Tyr		14.93 (1)	11.714 (9)	2.579 (1)	−7.753 (2)	−19.91 (4)		16.57 (1)		3.22	
L-Ala-L-Arg[c]			4.88 (6)	1.687 (2)	−7.584 (5)					3.19	

[a] Values in parentheses denote estimated standard deviations. [b] Taken from ref 8. [c] $\log \beta_{22-3} = -3.43$ (3).

Table III. Calculated Distributions of Deprotonated Monomeric and Dimeric Complexes in 1:1 Cu(II)–Dipeptide Systems[a]

dipeptide	pH	distribution, % monomer 5 mM	monomer 2 mM	dimer 5 mM	dimer 2 mM
L-Tyr-L-Arg	6.5	1	1	0	0
	9.5	47	56	30	17
	11.0	9	9	1	0
L-Tyr-D-Arg	6.5	0	0	0	0
	9.5	28	38	54	39
	11.0	8	8	4	2
L-Tyr-L-Tyr	6.0	0	0	0	0
	9.0	27	36	37	26
	10.3	10	14	6	4
	11.5	0	0	0	0
L-Tyr-D-Tyr	6.0	0	0	0	0
	9.0	42	49	19	10
	10.1	23	28	7	4
	11.5	0	0	0	0
L-Tyr-L-Trp	6.6	0	0	0	0
	9.5	13	19	81	71
	11.0	8	9	29	16
L-Try-D-Trp	6.6	1	0	0	0
	9.5	40	50	43	28
	11.0	10	10	3	1
L-Tyr-L-Phe	6.5	0	0	0	0
	9.5	51	61	34	20
	10.9	28	30	10	5

[a] The distributions were calculated from the stability constants listed in Table II and refer to the percentages based on total Cu(II).

Table IV. Visible Absorption Spectral Data for 1:1 Cu(II)–Dipeptide Systems at Neutral pH

dipeptide	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}
L-Tyr-Gly	7.4	632	85
L-Tyr-L-Ala	7.1	630	96
L-Tyr-L-Arg	7.5	628	98
L-Tyr-D-Glu	6.4	624	84
L-Tyr-L-His	9.0	586	64
L-Tyr-L-Tyr	6.1	628	89
L-Tyr-DTrp	6.2	619	84
L-Tyr-L-Trp	6.5	626	91
L-Tyr-D-Trp	6.5	624	106
L-Tyr-L-Phe	6.5	638	55
Gly-L-Tyr	7.0	635	82
L-Phe-L-Tyr	7.0	633	89
Gly-L-His	9.0	601	60

a positive peak at 660–680 nm at pH 6–11, while the active peptides with a C-terminal aromatic amino acid suffer a drastic spectral change with pH in the region 600–700 nm from the negative peak at pH <8 to the positive peak at pH 8–11 strictly concurrent with the dimer formation. In accordance with the earlier observation about Gly-L-Tyr,[8] L-Phe-L-Tyr gives no CT band at ~380 nm; at higher concentrations (>10 mM), however, Gly-L-Tyr was found to show the CT band as a weak shoulder. Although the equilibrium study failed to give the stability constants for the dimeric species in the Cu(II)–L-Tyr-L-/D-His systems, a definite CT peak was observed at pH >10 for Cu(II)–L-Tyr-D-His at 392 nm (ϵ 515) in the absorption and at 391 nm ($\Delta\epsilon$ −0.55) in the CD spectrum, indicating that the phenolate-bridged dimer is formed at higher pH than that for the other peptides.

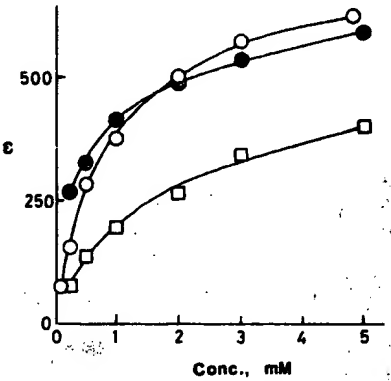

Figure 3. Concentration dependence of the 380-nm CT band intensity exhibited by 1:1 Cu(II)–dipeptide systems. Dipeptides: ○, L-Tyr-L-Ala; □, L-Tyr-D-Arg; ●, L-Tyr-L-Tyr.

Discussion

Effects of Peptides Side Chain on Deprotonation from Complexes. Tyrosine-containing peptide complexes can liberate protons from the peptide NH and phenol OH groups and the coordinated water. The pK_c values listed in Table II show that deprotonation from the peptide NH group is affected by the side groups. While the C-terminal aromatic ring apparently has no effect on pK_c, the N-terminal one lowers it as compared with the values for glycylglycine (4.10)[14] and Gly-L-Tyr. The charged groups on the C-terminal side chain, i.e., the guanidino group of L-Tyr-L-Arg and L-Ala-L-Arg and the carboxylato group of L-Tyr-L-/D-Glu, have a marked effect due t the charge of the resulting complex as a whole. Comparison of the log β_{110} values f r L-Tyr-Gly, Gly-L-Tyr, L-Tyr-L-Ala, L-Tyr-L-Arg, L-Tyr-L-Trp, and L-Tyr-L-Phe

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Table V. Absorption and CD Spectral Data for 1:1 Cu(II)–Dipeptide Systems at pH 9–10[a]

dipeptide	pH	conc, mM	absorption λ_{max}, nm (ϵ, M^{-1} cm^{-1})		CD λ_{max}, nm ($\Delta\epsilon$, M^{-1} cm^{-1})				I[d]
L-Tyr-Gly[b]	9.5	2.0	379 (385)	636 (107)	395 (–0.17)	548 (–0.08)	680 (+0.32)		var
L-Tyr-L-Ala[c]	9.8	2.0	376 (494)	626 (122)	393 (–0.31)	510 (–0.21)	618 (+0.06)		0.1
L-Tyr-D-Ala[c]	9.4	2.0	377 (270)	628 (100)	400 (–0.17)	493 (+0.10)	565 (–0.05)	670 (+0.64)	0.1
L-Tyr-L-Arg[c]	9.1	1.7	376 (372)	631 (102)	398 (–0.13)	508 (–0.24)	580 (–0.10)	685 (–0.37)	0.1
L-Tyr-D-Arg[c]	9.1	2.0	377 (258)	625 (104)	392 (–0.13)	498 (+0.12)	550 (+0.07)	662 (+0.82)	0.1
L-Tyr-D-Glu	9.4	5.0	378 (479)	623 (118)	402 (–0.16)	500 (+0.12)	555 (+0.04)	668 (+0.86)	0.1
L-Tyr-D-His	10.5	1.0	392 (515)	599 (120)	391 (–0.55)	534 (–0.26)	635 (+0.72)		var
L-Tyr-L-Tyr	9.5	2.0	384 (500)	632 (129)	388 (–0.49)	503 (–0.23)	574 (–0.29)	686 (+0.28)	var
L-Tyr-D-Tyr	9.5	2.0	384 (425)	620 (108)	395 (–0.61)	490 (+0.10)	657 (+1.00)		var
L-Tyr-L-Trp	9.5	2.0	382 (716)	628 (150)	383 (–0.78)	508 (–0.32)	568 (–0.34)	678 (+0.78)	var
L-Tyr-D-Trp	9.5	2.0	382 (579)	610 (114)	395 (–0.37)	505 (+0.15)	655 (+0.91)		var
L-Tyr-L-Phe	9.4	2.0	380 (506)	630 (119)	392 (–0.27)	508 (–0.24)	690 (+0.04)		var
Gly-L-Tyr	10.0	2.0		634 (81)		520 (–0.21)	663 (–0.84)		var
L-Phe-L-Tyr	10.0	2.0		631 (89)		520 (–0.22)	660 (–0.75)		var

[a] The data for L-Tyr-D-His were taken at pH 10.5 because of the dimer formation at high pH. [b] For the isolated crystals $Na_2[Cu_2(L\text{-}Tyr\text{-}Gly)_2]\cdot 10.5H_2O$ dissolved in water (pH 9.8), the λ_{max} (ϵ) values are the following: 382 (630), 639 (138). [c] The CD spectra were measured for 5 mM solutions. [d] The ionic strength varied from 0 M to 0.5 M ($NaClO_4$) has negligible effects on the spectra for L-Tyr-Gly and L-Tyr-L-Tyr except for a decrease (0.04) of the 686-nm CD peak for the latter.

indicates that the stability difference of the deprotonated complexes 1 due to the C-terminal side group is rather small (<0.65), so that it is difficult to assess the direct influence of the side groups on the complex stability. As previously discussed,[8] the second deprotonation can occur from the phenol OH group or the coordinated water molecule and is probably the mixture of the two steps, since dissociation of the phenol should be necessary for the dimer formation, although the macroscopic dissociation constants given by $\log \beta_{110} - \log \beta_{11-1}$ are closer to the values (9.3–9.4)[14a,15] for dissociation of the coordinated water. The microscopic pK_a values for Tyr-Gly have been calculated to be 8.42 and 9.90 for species H_2L and HL, respectively, as the phenol deprotonation step.[16]

Formation of Phenolate-Bridged Dimers. In aqueous solution the phenol moiety of tyrosine is not involved in the bonding with Cu(II) under normal conditions.[17] From the solution equilibrium study, the dimers have been found to be formed by the dipeptides with N-terminal tyrosine employed in the present study at pH 8–11 with the exception of Tyr-His. The appearance of the 380-nm absorption peak which accompanies the dimer formation indicates that the phenolate–Cu(II) bond serves as a bridge connecting the two Cu(II) complex units, because the peak at 380–400 nm has been known to be characteristic of the phenolate–Cu(II) bonding in several tyrosine-containing copper(II) complexes.[8,18-22] Direct evidence for the phenolate–Cu(II) bonding is given by the resonance Raman spectra of the monomeric and dimeric complexes of L-Tyr-Gly, $[Cu(L\text{-}Tyr\text{-}Gly)]\cdot 0.5H_2O$, and $Na_2[Cu_2(L\text{-}Tyr\text{-}Gly)_2]\cdot 7.5H_2O$, which were isolated as crystals (Figure 4). The spectra of the dimeric complex in the region 300–1700 cm^{-1} measured upon excitation into the 380-nm absorption envelope with 457.9-, 476.5-, and 514.5-nm Ar^+ lasers exhibit resonance-enhanced bands at 1170, 1264, 1501, and 1603 cm^{-1}. The spectral feature is very similar to that observed for the Cu(II)–*o*-tyrosine[21] and Cu(II)–transferrin[23,24] complexes, and all the bands are due to the phenolate moiety: the

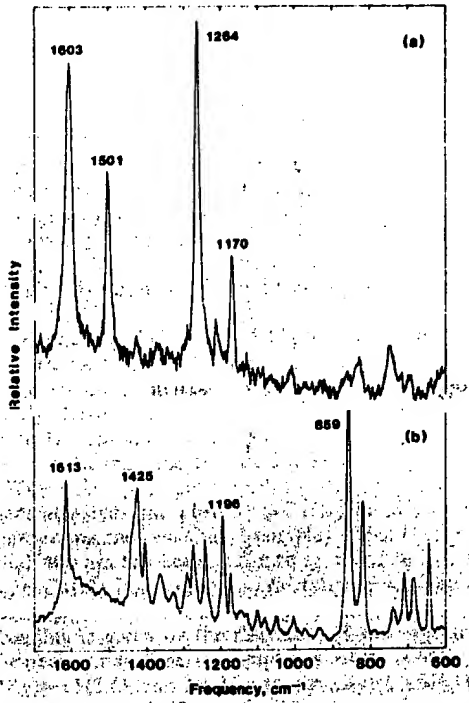

Figure 4. Resonance Raman spectra of $Na_2[Cu_2(L\text{-}Tyr\text{-}Gly)_2]\cdot 7.5H_2O$ (a) and $[Cu(L\text{-}Tyr\text{-}Gly)]\cdot 0.5H_2O$ (b). Conditions: KBr disk method; 457.9-nm Ar^+ laser excitation; slit width, 5.0×10^{-2} cm.

bands at 1501 and 1603 cm^{-1} are assigned to the C–C stretching modes of the ring, the band at 1264 cm^{-1} to the C–O stretching, and that at 1170 cm^{-1} to the C–H deformation.[23,24] The spectra for the monomeric complex, on the other hand, exhibit no resonance-enhanced bands in the same region, which is in accordance with the lack of the 380-nm absorption peak. These results, together with the electronic spectra and the elemental analyses of the two complexes, establish that the dimer is formed through the phenolate–Cu(II) bridges and that the 380-nm band is due to the CT transition involving Cu(II) and the phenolate oxygen.

In contrast with the CD spectra, the absorption spectra in the d–d region are little affected by the dimer formation except for the increase in intensity; a 5 mM solution of 1:1 Cu(II)–L-Tyr-L-Tyr has the λ_{max} (ϵ) values of 628 (89) and 632 (129) at pH 6.1 and 9.5, respectively, and a 2 mM solution of 1:1 Cu(II)–L-Tyr-L-Trp 626 (91) and 629 (150) at pH 6.5 and 9.5, respectively. The calculated percentage distributions (Table III) clearly indicate the dimer formation by the peptides with a C-terminal aromatic ring is favored for the active peptides especially

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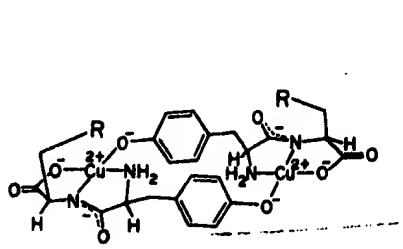
with tryptophan, which corresponds well with the CT band intensity at ~380 nm (Table V). In view f the stability order and conformational change to be discussed bel w, aromatic ring stacking at the C-terminus in the dimeric structure may be responsible for this trend.

On the basis f molecular models, Hefford and Pettit proposed an extended structure **2** for the dimer, where stacking between the two bridging phenolate rings was considered to serve as a stabilizing factor.[8] On the other hand, it is also possible to



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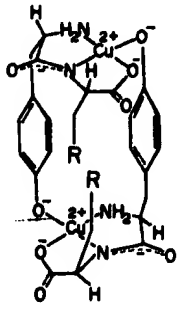

3

construct a folded structure **3**, which may be stabilized by the stacking between the two parallel phenolate rings and more importantly by additional stacking between closely situated aromatic rings when R is aromatic. The equilibrium between the monomeric and dimeric species is expressed by

$$2CuLH_{0(or\ -1)} \rightleftharpoons Cu_2L_2H_{0(or\ -2)} \qquad (4)$$

where log K_D is given by log $\beta_{22(or\ -2)}$ − 2 log $\beta_{110(or\ -1)}$ (Table II). For Tyr-Tyr and Tyr-Trp diastereomers, the active peptides have higher log K_D values than those for the meso peptides. This can be accommodated by structure **3**, where the C-terminal indole and phenol groups may stabilize the active (but not the meso) complexes by stacking interactions. The order of log K_D values for the peptides with aliphatic or charged groups, on the other hand, is not straightforward; because of steric hindrance and/or electrostatic repulsion the active complexes might prefer structure **2**, while the meso complexes might still prefer **3** where the side groups are directed toward the outside of the domain produced by the folded dimeric core. As described in the Results section, Gly-L-Tyr appears to form the dimer at high concentrations, but its distribution is very limited probably because of the high pK_a values for the phenol OH groups (log β_{11-1} − log β_{11-2}) in the complex and the steric crowdedness both in the extended and the folded structure with the two Cu(II) centers too close to each other.

CD Magntiude Anomaly Caused by Dimer Formation and Its Structural Implications. The CD spectra of the active complexes in the region 500–800 nm undergo a remarkable magnitude change and sign inversion accompanying the dimer formation at pH >8. That the spectral changes result from the dimer formation is evidenced by the effects of concentration and ammonia on the absorption and CD spectra of 1:1 Cu(II)–L-Tyr-L-Tyr at pH 9.5 (Figure 5); the CT band disappears with a concomitant shift of the d–d peak from 634 to 612 nm due to the 4N chromophore, and the CD spectrum resumes its pattern observed at pH <8 and >11.

For copper(II) and nickel(II) complexes of simple di- and tripeptides, the CD mangitudes in the d–d region have been found to be an additive function of the values for the complexes of the component amino acid residues.[5,25] Thus, the estimated magnitude $\Delta\epsilon_{calcd}$ for the 1:1 copper(II) complex of a peptide X-Y is given by

$$\Delta\epsilon_{calcd} = \Delta\epsilon_{Cu(X\text{-}Gly)} + \Delta\epsilon_{Cu(Gly\text{-}Y)} \qquad (5)$$

where $\Delta\epsilon_{Cu(X\text{-}Gly)}$ and $\Delta\epsilon_{Cu(Gly\text{-}Y)}$ denote the magnitudes exhibited by the 1:1 complexes f X-Gly and Gly-Y, respectively. The $\Delta\epsilon_{calcd}$ values at 600–700 nm are shown in Table VI together with the differences $|\Delta\epsilon - \Delta\epsilon_{calcd}|$ which serve as a criterion of the magnitude additivity. While the additivity in the d–d region holds for the complexes of the peptides with a C-terminal aliphatic amino acid or a D-aromatic amino acid over a wide range of pH, a surprisingly large deviation from $\Delta\epsilon_{calcd}$ with sign inversion occurs at pH 8–11 with the peak at 600–700 nm for the active complexes comprising a C-terminal L-aromatic amino acid, i.e., L-Tyr-L-Tyr, L-Tyr-L-Trp, and L-Tyr-L-Phe. The magnitude anomaly which is diastereospecific is ascribed to the dimers, because it disappears at low concentrations (<0.2 mM), at pH <8 and >11, or in the presence of ammonia. The systems with L-Phe-L-Tyr gives normal $\Delta\epsilon$ values at pH 8–11, indicating that negligible anomaly arises from two aromatic rings situated on the same side of the copper(II) coordination plane unless the dimer is formed.

The CD spectra in the d–d region observed for the Cu(II)–dipeptide complexes reflect the peptide side-chain conformations. Anomalous CD magnitudes have been reported for the ternary Cu(II)–amino acid systems involving an aromatic amino acid such as phenylalanine[17,26] and for those involving an acidic and a basic amino acid whose oppositely charged side chains interact with each other electrostatically and thus become fixed around copper(II).[27,28]

Table VI. CD Magnitude Additivity in the Region 600–700 nm in 1:1 Cu(II)–Dipeptide Systems[a]

dipeptide[b]	pH	λ_{max}, nm	$\Delta\epsilon$	$\Delta\epsilon_{calcd}$[d]	Δ[e]	sign inversion
L-Tyr-L-Ala	6.4	680	−0.30	−0.19	0.11	no
	9.8	618	+0.06	+0.01	0.05	no
	11.2	595	+0.10	+0.09	0.01	no
L-Tyr-D-Ala	6.2	652	+0.60	+0.51	0.09	no
	9.4	670	+0.64	+0.64	0.00	no
	11.4	675	+0.59	+0.52	0.07	no
L-Tyr-L-Arg	5.6	655	−0.50	−0.25	0.25	no
	9.1	680	−0.31	−0.22	0.09	no
	11.3	680	−0.36	−0.25	0.11	no
L-Tyr-D-Arg	5.6	645	+0.69	+0.59	0.10	no
	9.0	662	+0.82	+0.71	0.11	no
	11.2	658	+0.74	+0.66	0.08	no
L-Tyr-L-Tyr	6.1	647	−0.66	−0.52	0.13	no
	9.0	683	+0.39	−0.38	0.77	yes
	10.3	683	+0.35	−0.42	0.77	yes
	11.5	678	−0.59	−0.47	0.12	no
L-Tyr-D-Tyr	6.3	635	+0.90	+0.83	0.07	no
	9.0	662	+0.79	+0.87	0.08	no
	10.1	665	+0.85	+0.88	0.03	no
	11.5	668	+0.94	+0.86	0.08	no
L-Tyr-L-Trp[c]	6.6	636	−0.36	−0.40	0.04	no
	9.5	678	+0.78	−0.40	1.18	yes
	11.0	680	+0.11	−0.29	0.40	yes
L-Tyr-D-Trp[c]	6.6	634	+0.75	+0.55	0.20	no
	9.5	655	+0.91	+1.00	0.09	no
	11.0	663	+0.72	+0.69	0.03	no
L-Tyr-L-Phe[c]	6.5	642	−0.34	−0.30	0.04	no
	9.5	690	+0.04	−0.33	0.37	yes
	10.9	672	−0.41	−0.48	0.07	no
L-Phe-L-Tyr[c]	7.0	647	−0.74	−0.61	0.13	no
	10.0	667	−0.74	−0.68	0.06	no
	11.0	680	−0.66	−0.52	0.14	no

[a] Similar magnitudes were observed for L-Tyr-L-/D-Arg and L-Tyr-L-/D-Tyr in 50% aqueous ethanol (or methanol). [b] Measured at I = 0.1 ($NaClO_4$) at room temperature for 5 mM aqueous solutions. [c] Measured at I = var for 2 mM aqueous solutions. [d] Calculated according to eq 4. The values for the meso peptide complexes were estimated by assuming that 1:1 Cu(II)–Gly-D-X has a magnitude equal to that of 1:1 Cu(II)–Gly-L-X with the sign inversed. [e] $\Delta = |\Delta\epsilon - \Delta\epsilon_{calcd}|$.

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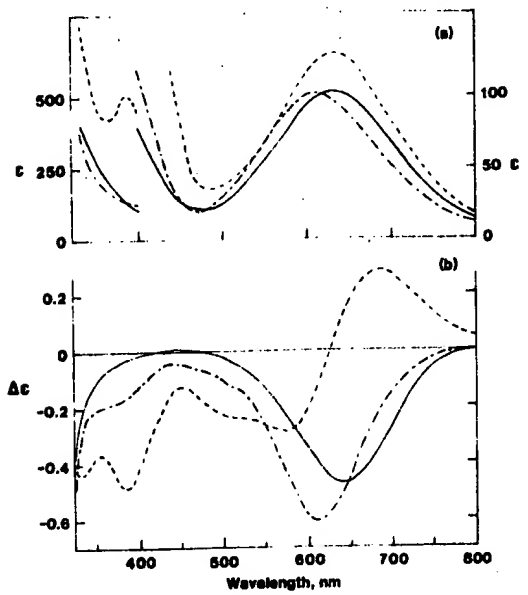
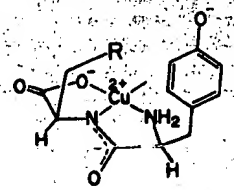


Figure 5. Absorption (a) and CD (b) spectra of 1:1 Cu(II)–L-Tyr-L-Tyr systems under various conditions. Curves: —, 0.2 mM; - - -, 2 mM; - · -, 2 mM in the presence of 4 mM NH_3. pH 9.5.

Regarding peptide complexes, the magnitude enhancement exhibited by Cu(II)– and Ni(II)–tetra- and pentapeptide complexes has been interpreted as due to intramolecular hydrogen bonding.[29] The present CD magnitude anomaly can be attributed to the conformational change of the C-terminal aromatic amino acid, because this is more influential on the spectral pattern[25,30] and its aromatic side chain prefers to be located above the metal coordination plane as in the Pd(II)–phenylalanylphenylalaninate complex in solution.[31] The latter point is further documented by the X-ray analyses of the copper(II) complexes of tyrosine,[32] glycyl-L-leucyl-L-tyrosine,[33] and glycyl-L-tryptophan[34] and the palladium(II) complex of tyrosine,[35] all of which have been disclosed to have the aromatic side group tilted toward the metal ions with the metal ion–aromatic ring separations (3.04–3.34 Å for the copper(II) complexes) smaller than the van der Waals contact without bonding through the phenol OH or indole NH group. We may then reasonably assume that the active monomeric complexes have a similar side-chain conformation **4**, which is



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distorted upon dimer formation by the bridging phenolate rings to make the side group lose access to the copper(II) coordination plane when the dimer has structure **3**. No conformational change should occur with the meso complexes because of the side chain directed outward, thus giving an explanation for the diastereospecificity of the magnitude anomaly. The aromatic ring stacking suggested by the stability constants for the active peptides with structure **3** makes the side-chain conformation more rigid, which is also compatible with the magnitude anomaly. It should be mentioned in this connection that ar matic ring stacking has a much greater stabilizing effect on the complex than the Cu(II)–aromatic ring interaction.[36,37]

Earlier observations of the CD magnitudes for the Cu(II)–aromatic amino acid complexes,[17,21,26] rotamer populations in Ni(II)–[38] and Pd(II)–peptide complexes[31,39-42] as calculated from NMR spectra, and ESR spectral parameters of tyrosine-containing ternary copper(II) complexes[43] have been taken to imply the metal ion–aromatic ring attractive interactions in solution, but more convincing information concerning copper(II) complexes has been lacking. The magnitude anomaly observed for the present systems in turn strongly supports the preferred orientation of the side-chain aromatic ring above the coordination plane of monomeric complexes of peptides, because the actual magnitudes used in eq 5 refer to the tilted or *normal* conformations in monomeric complexes, which will not be preserved in structure **3** owing to steric hindrance.

Conclusion. The side chains of L-Tyr-containing dipeptides have rather small influences on the solution equilibria and the spectra of the copper(II) complexes in acid–neutral solution, where the side-chain orientations are free from steric hindrance. The resonance Raman spectra of the isolated monomeric and dimeric complexes confirm both the existence of the phenolate–Cu(II) bond and the assignment of the 380-nm absorption peak to the CT transition involving this bond. The information therefrom supports well the interpretation of the structures and side-chain conformations of the complexes in solution. Thus, the remarkable CD spectral anomaly observed with formation of the dimeric complexes is reasonably taken to indicate the close contact between Cu(II) and the aromatic ring in the absence of steric hindrance and further support the hitherto proposed cases of Cu(II)–aromatic ring interactions in similar complexes.[17,21,26,43]

The roles of copper(II) in brain may be seen from the fact that copper deficiency in humans is symptomized by tremors or seizures and that an epileptic patients' brain has markedly decreased copper concentrations.[44,45] A recent study of the Cu(II)–enkephalin (methionine–enkephalin) complex showed that it is slightly more potent than the enkephalin itself.[46] The present investigation shows that the endogenous analgesic dipeptide kyotorphin and other tyrosine-containing dipeptides readily form copper(II) complexes, where the phenol group can be involved in the copper(II) binding and the side-chain aromatic group is probably located close to the coordination plane. As a result of copper(II) complex formation, the analgesic activity of opioid peptides could be modulated by the binding modes and the side-chain conformations in the complexes.

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Kinetics of the Reactions of (Ethoxycarbonyl)methylcobalt Tetracarbonyl with ^{13}CO, Ph_3P, $HCo(CO)_4$, and H_2. A Comparison of the Reactivities of $RCo(CO)_4$ (R = CH_2COOEt, COOEt, and H) Complexes

C. D. Hoff,*† F. Ungváry,*‡ R. B. King,*§ and L. Markó*‡

Contribution from the Department of Chemistry, University of Miami, Coral Gables, Florida 33124, the Institute of Organic Chemistry, University of Chemical Engineering, H-8201 Veszprém, Hungary, and the Department of Chemistry, University of Georgia, Athens, Georgia 30602. *Received October 3, 1983*

Abstract: The kinetics of the reactions of (ethoxycarbonyl)methylcobalt tetracarbonyl (**1**) with ^{13}CO, Ph_3P, $HCo(CO)_4$, and H_2 are consistent with initial reversible dissociation of $EtOOCCH_2Co(CO)_4$ (**1**) to $EtOOCCH_2Co(CO)_3$ and CO. The alkylcobalt tricarbonyl then reacts competitively with the other reaction partner. The relative reactivities of **2** toward Ph_3P, CO, $HCo(CO)_4$, and H_2 are 1.82, 1.0, 0.078, and 0.0006 at 25 °C in *n*-heptane. The rate of ^{13}CO substitution has also been measured for $HCo(CO)_4$, $Co_2(CO)_8$, and (ethoxycarbonyl)cobalt tetracarbonyl (**2**). The half-life for $HCo(CO)_4$ at −30 °C is 45 s, $t_{1/2}$ for $EtOOCCH_2Co(CO)_4$ at 15 °C is 18.8 min, and $t_{1/2}$ for $EtOOCCo(CO)_4$ (**2**) at 15 °C is 24.2 min. The complex **2** shows a slow ^{13}CO incorporation into the acyl carbonyl group, $t_{1/2} \approx 50$ h at 28 °C, presumably through an ethoxycobalt tetracarbonyl intermediate. The rate of ^{13}CO exchange with $Co_2(CO)_8$ is not influenced by the presence of $HCo(CO)_4$ at 0 °C in *n*-octane, indicating that exchange of cobalt centers between $HCo(CO)_4$ and $Co_2(CO)_8$ is slow compared to carbonyl exchange.

Recent work has shown that bimolecular reductive elimination between a transition-metal alkyl or acyl and a transition-metal hydride can proceed through several different mechanisms.[1] These reactions are central to a number of catalytic cycles, particularly hydrogenation and hydroformylation. Although cobalt-catalyzed hydroformylation has been widely studied, there are few reports of the kinetics of isolated steps in this reaction.[2] In a preceding publication, two of us reported a kinetic study of the reactions of an acylcobalt tetracarbonyl, namely ethoxycarbonylcobalt tetracarbonyl, with Ph_3P, $HCo(CO)_4$, and H_2.[3]

No similar studies have been reported for alkylcobalt tetracarbonyls because they are difficult to obtain in pure form, owing to limited stability in most cases.[4] Galamb and Pályi reported recently[5] the isolation of a series of cobalt alkyls $ROOCCH_2Co(CO)_4$ from the reaction of $NaCo(CO)_4$ and $ROOCCH_2X$. Using this possibility, we now report details of our investigations on a cobalt alkyl analogous to those reported earlier for the cobalt acyl.

In this study, as in the proposed mechanism for hydroformylation, coordinatively unsaturated species play a prominent role. While there is evidence for the existence of $HCo(CO)_3$,[6] there is no information regarding the rate of formation of this compound or analogous alkyl and acylcobalt tricarbonyls. We also report measurements of the rates of ^{13}CO exchange for each of these species. These data provide support for our proposed mechanism and furnish fundamental information relevant to the hydroformylation reaction.

Results

Reaction of $EtOOCCH_2Co(CO)_4$ (1) with ^{13}CO. The ^{13}CO exchange reaction (eq 1) could be followed by measuring the

$$EtOOCCH_2Co(CO)_4 + {}^{13}CO \rightarrow EtOOCCH_2Co(CO)_3({}^{13}CO) + CO \quad (1)$$

decrease of absorbance of the A_1 band of **1** at 2111.5 cm^{-1} under 1 atm pressure of carbon monoxide enriched to 86% in ^{13}CO. Reported half-lives and rate constants for all ^{13}CO reactions have been corrected for work under 100% isotopic enrichment using standard procedures.[7]

Table I. Half-Life Times of CO Exchange with ^{13}CO ($t_{1/2}$) for $RCo(CO)_4$ Complexes in *n*-Heptane Solution and the Calculated First-Order Rate Constants ($k'_1 = (\ln 2)/t_{1/2}$)

R	temp, °C	$t_{1/2}$, min	$10^5 \times k'_1$, s^{-1}
$(OC)_4Co$	0	27.4	42[a]
	0	27.4	42[a,b]
	0	38.5	30[c]
	0		41[d]
H	−30	0.75	1540
$EtOOCCH_2$	25	4.3	268
	15	18.8	61.4
	5	70	16.5
EtOOC	~28	~3000	0.4[e]
	15	24.2	47.3

[a] Refers to the overall rate of ^{13}CO incorporation. Determined as described in ref 10b. [b] In the presence of 5 mol % $HCo(CO)_4$. [c] For the reaction of $Co_2(^{13}CO)_8$ (86% isotope purity prepared in situ by previously equilibrating $Co_2(CO)_8$ with excess ^{13}CO with ^{12}CO in the presence of 330 mol % $HCo(CO)_4$. [d] Reference 10b. [e] Refers to the ^{13}CO exchange of the acyl carbonyl group.

† University of Miami.
‡ University of Chemical Engineering.
§ University of Georgia.

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